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Faculty of Pure and Applied sciences

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bachelors in parasitology about:**

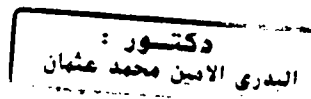
**In vitro antitrichomonas activity
of lowsonia inerimtes – activity**

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الإهداء

إلى أعزة كانوا مطشاعرنا شموخا وعزا ارتقي حياتنا

في طريق العلم درجات لا تقدر بثمن

وأعطوا وثقافوا وأوفوا بالقسط

وأغروا العين بهاءا و عطاءا

بلا حدود

قال ثم من،،، قلت أمي

قال ثم من قلت طيبة القلوب

قال ثم من قلت :

حليمة حمد محمد

و إلى من كان سببا في حياتي وجرأ من العطف

السلام

ما عطشت يوما إلا وارتويت من حكمته و صبره

و لا أواني أن أقدمه على الرجال

شرفا وحسنا وحبا

أبي الغالي قائد لواء التربية

علي آدم علي

ورفيقة دربي

إخواني أساتذتي ،، زملائي أصدقائي

لكم جميعا أهدي بحبي

الباحث

شكر و عرفان

الشكر من قبل ومن بعد لله رب العالمين لاتمام هذا البحث
ثم الشكر موصول إلى كل من ساهم في جمع مادة هذا البحث
وأخص بالشكر الدكتور: **البكري محمد الأمين عثمان** .
الذي أولى هذه الدراسة الإهتمام والعناية واهداني بكل ما تحتاجه
الدراسة فكان حينئذ مرشد ودليل كما الشكر لكل العاملين لمركز
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المبحث

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CHAPTER ONE
INTRODUCTION
&
LITERATURE REVIEW

1.1 Introduction:

Trichomonas vaginalis infection is one of the major health problems in the world, and the most common transmitted infections in many regions including the developed countries such as United State (Parbara ,Van Der Pol, 2005). *Trichomonas vaginalis* infection should not be considered as a simple vaginal infection that serves markers for others sexually transmitted diseases in recent years. Moreover, *Trichomonas vaginalis* infection have been associated with several adverse outcomes including adverse pregnancy, pelvic inflammatory premature delivery, , infertility, postoperative infections, low birth weight , cervical neoplasia and increased susceptibility to HIV infection (Schwebke, *et al* 1997).

Medicinal plants are still invaluable source of safe, less toxicity, lower price, available and reliable natural resources of drugs all over the world, despite the bulk work which have been done in this concern by Sudanese researcher; it is undoubtedly that this field remains rich and not wholly exploited (Gamal, *et al* 1994).

Thus the need of alternative drugs to reduce their burden of purchasing the synthetic drugs especially after the problem of getting resistant to many clinical patients against metronidazole (Iran, *et al* 2006; Pratibha *et al*, 2008). In addition, metronidazole sometimes causes adverse effects, e.g., myopia, neuralgia, and allergic dermatitis (Upcrof *et al*, 2006), and thus new anti-trichomonal drugs are probably required.

Sudan is rich in medicinal flora with antiprotozoal, antimicrobial and antifungal activities like *Acacia nilotica* (EL-Kamali, *et al* 1996).

Objectives of the study:

- 1- Investigate Sudanese plants of *Lawsonia inermis*
- 2- Compare *in vitro* the most active effect of the mentioned plant with metronidazole a synthetic drug used for treatment of trichomoniasis.

1.2 Literature review:

1-2-1 Historical back ground:

Trichomonas vaginalis is a parasitic protozoan that is the cause of trichomoniasis, a sexually transmitted disease (STD) of worldwide importance. Although the disease was first described as a clinical entity by Hohne in 1916 (Hohne, 1916), the etiological agent, *T. vaginalis*, was first identified by Donne' who visualized motile microorganisms in the purulent, frothy leukorrhea of women presenting with vaginal discharge and genital irritation (Petrin, *et al*, 1998). In 1916 Hohne used the term trichomoniasis to describe the condition where in the vagina contained many of the flagellates. However, research on this organism did not begin until the 20th centuries. In the 1960s and 1970s, the research focused on biochemical tests and microscopic examination to understand the growth characteristics and behavior of the organism. It was not until the 1980s that immunologic methods and molecular biology techniques became available and were applied to study the pathogenesis and immunology of this organism.

1.2.2 Classification:

T. vaginalis is a parasitic protozoan, and the taxonomic position is based on the classification scheme by (Dyer, 1990), in which protozoa with the "9 + 2" flagellum fall into the phylum Zoomastigina.

- Phylum : Zoomastigina
- Class : Parabasalia
- Order : Trichomonadida
- Family : Trichomonadidae
- Genus : Trichomonas
- Species : *Trichomonas vaginalis*

(Donné, 1836).

1.2.3 Structure and life cycle and transmission:

The shape of *T. vaginalis* in culture is typically pyriform, although amoeboid shapes are evident in parasites adhering to vaginal tissues in vivo (nilsen, 1975). In axenic culture, the shape of the protozoan tends to be more uniform, i.e., pear shaped or oval (Arroyo, *et al* 1993).

T vaginalis is approximately the size of a white blood cell (about 10 µm in diameter), although its flagellum allows it to move around vaginal and urethral tissues (Forna, *et al*, 2003). The parasites takes on a more amoeboid appearance when attached to vaginal epithelial cells, and it has five flagella, four of which are located at its anterior portion that give the trichomonad its

characteristic jerky, swaying motion in wet mount. The fifth flagellum is incorporated within the undulating membrane of the parasite, which is supported by a slender non contractile costa. The flagella and the undulating membrane give this parasite characteristic quivering motility. In addition to the undulating membrane, one recurrent flagellum and the Costa originate in the kinetosomal complex at the anterior of the parasite. Internal organelles include a prominent nucleus and a rigid structure, the axostyle that runs through the cell from the anterior end to the posterior end (Honigberg, 1964; nilsen, 1975). Unique energy-producing organelles, the hydrogenosomes (Lindmark, 1979), are present as paraxostylar and Para costal chromatic granules by light microscopy and as osmiophilic granules by electron microscopy (Nilson, 1976). Under unfavorable growth conditions, *Trichomonas vaginalis* can round up and internalize the flagella.

The life cycle of *T. vaginalis* is simple in that the trophozoite is transmitted through coitus and no cyst form is known (the trophozoite is transmitted from one person to another, usually by sexual intercourse.). The *trophozoite* divides by binary fission and, in natural infections, gives rise to a population in the lumen and on the mucosal surfaces of the urogenital tracts of humans (Jane, 2004).

Four points support the belief that *T. vaginalis* is transmitted sexually. The most important evidence is the high rate of infection of the urethra and/or prostate in the male partners of infected females. In such cases, recurrent

Trichomonal vaginitis was cured only after the parasites had been eradicated from the genital tract of the patients' consorts. *T. vaginalis* is observed more frequently in females attending STD clinics and also in prostitutes than in postmenopausal women and virgins. Finally, the flagellates die outside the human body unless they are protected from drying (Catterall, 1972; Kaufman, *et al*, 1989, Krieger, 1981, Whittington, 1957).

Nonsexual transmission has been observed in cases such as by contaminated douche nozzles, specula, or toilet seats through which trichomonads may find their way into the vagina (Whittington, 1957). However, such cases are rare. Live *T. vaginalis* has been found in urine and in semen after several hours of exposure to air and in swimming pool water (Catterall, *et al*, 1960).

Newborn infants of mothers infected by *T. vaginalis* have on occasion acquired a *T. vaginalis* urinary tract or vaginal infection. The organisms were acquired by 2 to 17% of female neonates of infected women (al-Salihi, *et al* 1974).

T. vaginalis can persist for up to 5 years in women and is highly transmissible between partners (Bowden, *et al* 2000).

1.2.4.1 Epidemiology:

Trichomonal infection has been encountered in every continent and climate and has no seasonal variability. It has a cosmopolitan distribution and has been identified in all racial groups and socioeconomic strata. Recent data have shown that the annual worldwide incidence of trichomoniasis is more

than 250 million cases (Seema Sood, Arti Kapil. 2008).). In fact, the World Health Organization (WHO) has estimated that this infection accounts for almost half of all curable sexually transmitted infections (Cates, 1999; WHO, 2001).

As per the published literature, the prevalence estimates vary between population studied but range from .4 to 27.4% in women and 0.0 to 5.6% in men (Swygard *et al.* , 2004).

Trichomoniasis is the most common nonviral sexually transmitted disease. *T. vaginalis* infections are commonly associated with other sexual transmitted diseases (STDs) and are a marker of high-risk sexual behavior. Trichomoniasis is frequently seen concomitantly with other STDs, particularly gonorrhea (Lossick, 1989).

The majority of women with trichomoniasis also have bacterial vaginosis (Jemes , 1992; Thomason, 1988). Unlike other STDs, which have a higher prevalence among adolescents and young adults, the rates of trichomoniasis are more evenly distributed among sexually active women of all age groups, further strengthening its potential utility as a marker for risky sexual behavior (Lossick, 1989).

Although survival on fomites is documented, the organism is thought to be transmitted almost exclusively by sexual activity (Wilcox, 1960). Humans are the only natural host for *T. vaginalis*. The incubation period of the disease is typically 5-28 days. The trophozoite is transmitted from person to

person through sexual intercourse. Nonsexual transmission of the disease is rare (Petrin et al, 1998). Asymptomatic infection rates are as high as 50% in women. Infected men are usually asymptomatic and the infection is usually self-limiting; diagnosis is thus often difficult (Petrin *et al*, 1998).

1.2.6.1 Pathogenesis:

The parasite infection has been associated with *vaginitis*, *urethritis*, *prostatitis*, *epididymitis*, cervical cancer, infertility, and pelvic inflammatory disease (PID).

The most important clinical signs of trichomoniasis are vaginal or urethral discharge, foul-smelling discharge, dysuria, puritus, severe irritation, abdominal pain, and edema or erythema (Swygard *et al*, 2004; Mundodi *et al*, 2006).

This discharge is frequently profuse and is often associated with burning, itching, or chafing. When viewed through a speculum, the vaginal mucosa is sometimes diffusely hyperemic, with bright red punctuate lesions, although sometimes it is only patchily hyperemic and not infrequently normal in appearance.

The frequency of urination and dysuria are the commonest associated symptoms, and urethral involvement is found in a large proportion of cases (Markell *et al*, 1999)

1.2.6.2 Factors involved in the pathogenesis of *T. vaginalis*:

The factors considered to be involved in the pathogenicity of *T. vaginalis* include; the ability of trichomonads to adhere to vaginal epithelial cells (VECs), the cytotoxic effect of the pathogen on host cells, trichomonad proteinase activity, and the ability of trichomonads to produce subcutaneous abscess lesions (Alderete and Pearlman, 1984; Alderete and Garza, 1985; Alderete *et al*, 1995; Krieger *et al.*, 1985).

1.2.8.1 Diagnosis:

The classic symptoms associated with infection by *T vaginalis* include a yellowish-green frothy discharge, pruritis, dysuria, dyspareunia, and the 'strawberry cervix, which is characterized by punctate hemorrhagic lesions.

Diagnosis of trichomoniasis has traditionally depended on the microscopic observation of motile protozoa in vaginal or cervical secretions, a procedure first described by Donné in 1836 (Donné, 1836).

Various methods have been used to diagnose trichomoniasis, such as, wet mount, culture, Papanicolaou smear, serologic tests and DNA techniques.

1.2.8.2 Saline microscopic examination:

Vaginal trichomoniasis is typically diagnosed with microscopy. A vaginal swab sample for saline wet mount evaluation is an easy, valuable, and economical tool, but specificity is limited and the slide should be evaluated

immediately. Microscopy yields a sensitivity of 60-70% in the detection of *T vaginalis* in vaginal secretions (Workowski *et al*, 2006).

The absence of trichomonads on microscopy does not exclude the possibility of trichomoniasis (D Scott, 2008).

1.2.8.3 PH testing:

In women with trichomoniasis, the pH of vaginal secretions measured on Nitrazine paper is often elevated (>4.5). However, an elevation in pH is not highly specific. Bacterial vaginosis frequently also elevates the pH (Workowski *et al*, 2006).

Upon application of 10% potassium hydroxide to a vaginal swab sample in the potassium hydroxide (KOH) amine test, a fishy odor is released, which can suggest trichomoniasis or bacterial vaginosis.

12.8.4 Standard culture:

Culture is more sensitive and specific than microscopy (Workowski *et al*, 2006). Culture yields a sensitivity of about 95 % (Patel *et al*, 2000).

The broth culture method is the "gold standard" for the diagnosis of trichomoniasis because it is simple to interpret and requires as few as 300 to 500 trichomonads/ml of inoculum (Garber *et al*, 1987) to initiate growth in culture. However, there are inherent limitations to culture diagnosis (Farris *et al*, 1970).